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IN ADENOVIRUS TYPE 12-INDUCED HAMSTER SARCOMA CELLS

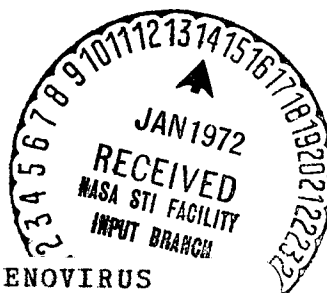
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GLYCOLYSIS IN ADENOVIRUS-INFECTED RAT CELL CULTURES AND  
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I. Ya. Kogan, A. N. Saprin<sup>1</sup>

ABSTRACT: It was demonstrated that in cultures of fibroblasts of rat embryos (REF) infected with human adenoviruses, statistically reliable ( $p \geq 0.001$ ) increase of glycolytic reactions in contrast with the control, depending on types of inoculated viruses were observed. Cultures of REF cells, infected with oncogenic adenovirus, type 12, were characterized by more pronounced aerobic and anaerobic glycolysis than REF cultures infected with adenovirus, types 3 and 6. Cells of rat fibroblasts infected with human adenovirus utilize glucose under aerobic conditions more intensively in comparison with control ones. There were noted identical shifts in glycolytic reactions in REF cell cultures infected with adenovirus, type 12, and in cells of hamster sarcoma A-12 cultivated in vitro.

An essential stage of cancer development is the change in cellular energy metabolism toward a so-called "cancerous stereotype" in which an important role belongs to glycolytic processes. But, at the present time, the question of at what moment the cell becomes malignantly transformed, i.e., when it acquires biological properties characteristic of tumor tissue, is poorly studied. There are only individual references to the research in this direction. /49\*

D. B. Golubev and O. A. Mil'Stein [1, 2] noticed that all the indices of carbohydrate metabolism, including the pathology of the isozyme spectrum of lactate dehydrogenase in the culture of fibroblasts of hamster embryos, were more marked in cultures infected with the strongly oncogenic adenovirus type 12 than in cultures infected with adenovirus type 1 [1, 2]. Porwit-Bohr [5] also discovered limitation of endogenous respiration and increase in aerobic glycolysis by embryonal cells of mice infected with polyoma virus, which in

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\* Numbers in the margin indicate pagination in the foreign text.

the author's opinion is one of the early symptoms in the disturbance of metabolism, inherent in the malignantly transformed tissue.

Starting out from the above-exposed facts, a comparative study of certain indices of carbohydrate metabolism in the cell at its initial contact with infectious and oncogenic viruses would present a definite interest.

The present work is devoted to the study of aerobic and anerobic glycolysis in the culture of fibroblast cells of rat embryos (REF) infected with human adenoviruses: with the strongly oncogenic variant type 12, with the weakly oncogenic variant type 3, and with the non-oncogenic infective variant type 6, and to the study of glycolysis in the culture of sarcoma cells of hamsters in which the sarcoma was induced by adenovirus type 12.

Materials and Methods. For the studies we used single layer cultures of rat embryo fibroblasts (REF); the cultures were obtained by the generally accepted methodology [4]. The initially trypsinized cultures were cultivated in 1.5 liter ROUX separating flasks in No. 199 growth nutrient medium with 2% native bovine serum. Three to four days after the formation of a monolayer, the cells were inoculated with adenoviruses type 3, 6, and 12, which passed in a CaVe tissue culture in a titer of  $10^{2.5-4.0}$  TPD/1 ml. At the same time, the media were changed in the control tests. The infection was repeated three times at one hour intervals. The virus reproduction was studied by the increase in infectious titer in the REF culture. Examination was made every hour for the first six hours, then every six hours for the first day, and then daily for 7 days after the infection of the REF tissue culture with adenoviruses. The cellular monlayer was removed with 0.02% Versen solution, and resuspended in No. 199 medium. Of the cellular suspension, 1.5 ml (15-20 million cells) were introduced into a Warburg flask, which contained 0.5 ml of 0.02 M glucose, and 1 ml of 0.03 M ATP<sub>H</sub>, and incubated for one hour in the Warburg apparatus. For the creation of anaerobic conditions, the tests were conducted in nitrogen atmosphere; for aerobic conditions, the air was the gaseous medium.

The intensity of glycolysis was estimated by the utilization of glucose, and by the increase in the lactic acid end product. Glucose was determined by the highly specific enzymatic method with glucose-oxidase, and the lactic acid with p-oxydiphenyl [3].

For control, we used a normal non-infected REF culture, as well as a culture <sup>50</sup> of rat fibroblasts infected with aviral CaVe extract, and with viruses which were inactivated at 56°C for one hour. The cultures of the sarcoma cells of hamsters which were induced with adenovirus type 12 were obtained by way of initial trypsinization. A-12 sarcomas developed in 60-70% of Syrian hamsters 30 to 40 days after their inoculation in the newborn state with adenovirus type 12 in a titer of  $10^{5.5}$  TCPD/ml.

In all tests, the infectious titer of viruses was determined at different times after inoculation by destroying the cells with triple freezing and thawing. The infectious titer was taken into account according to the results of an interaction of the adenoviruses with a susceptible culture of human embryonal kidneys and with CaVe culture.

The results, presented below, were obtained in ten tests of the same type.

Results and their discussion. The studies showed that the infection of the initially trypsinized culture of rat embryo fibroblasts (REF) with adenoviruses types 3, 6, and 12 resulted in strengthening both anaerobic and aerobic glycolysis. Such an increase in the glycolytic reactions was however observed just 2-4 hours after the introduction of viruses into the REF culture; it depended upon the types of the inoculated viruses, and it was statistically significant ( $P \leq 0.001$ ) when compared with the control (Figure 1).

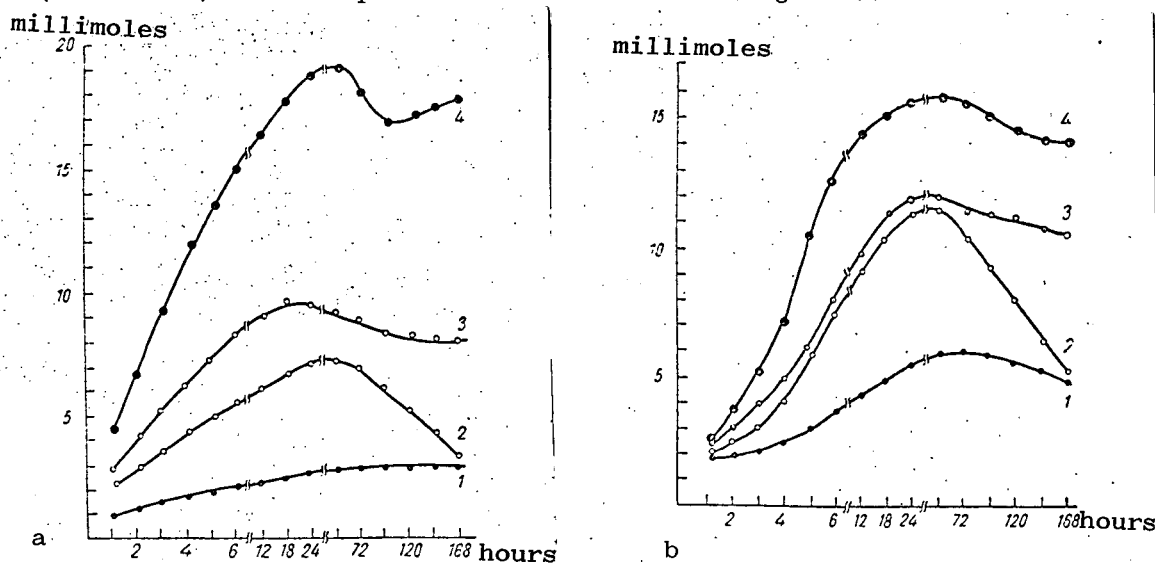


Figure 1. Aerobic (a) and anaerobic (b) glycolysis in REF tissue cultures infected with adenoviruses.

1- control; 2- type 6 adenovirus; 3- type 3 adenovirus; 4- type 12 adenovirus. On ordinate - lactic acid (in millimoles); on abscissa - hours after infection (for all plottings).

It is important to mention that REF cultures infected with type 12 oncogenic adenovirus had more marked aerobic and anaerobic glycolysis than REF cultures infected with type 3 and type 6 viruses. Moreover, the increase in the intensity of glycolytic reactions remained monotonous when in the REF tissue culture the oncogenic variant type 12 and the weakly oncogenic variant type 3 were introduced, although as with the inoculation of the infective variant type 6 of the adenovirus, a reinforcement of glycolysis was observed only until 48-72 hours with subsequent drop. It is probable that this is conditioned by a specific interaction of human adenoviruses with embryonal fibroblasts of rats. /51

The infective titer of adenoviruses type 3 and type 6 increased from the first hour after their inoculation into the REF tissue culture cells, reaching a maximum after 72 hours, although the titer of adenovirus type 12 did not increase (Figure 2). It should also be mentioned, that the number of cells gradually decreased in the culture infected with adenovirus type 6, while in the REF cultures infected with adenoviruses type 3 and type 12, no noticeable decrease was observed in the number of cells in the monolayer (Figure 3).

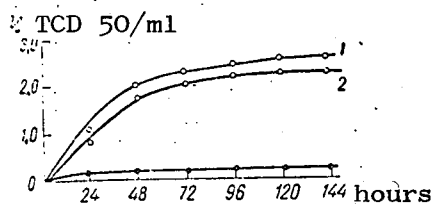


Figure 2. Development of the change in infectious titer of viruses in the REF culture.

1- adenovirus type 6; 2- adenovirus type 3; 3- adenovirus type 12. Ordinate: TCD 50/ml; abscissa: hours after infection.

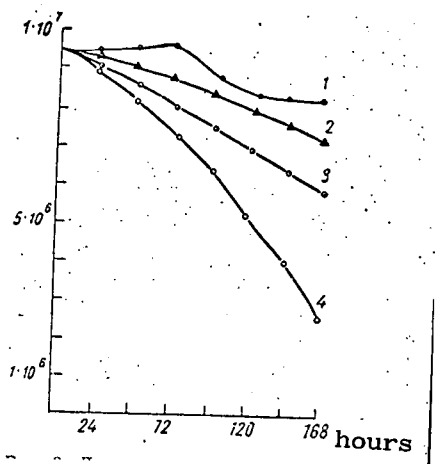


Figure 3. Development of the change in cell number in the REF cultures infected with adenoviruses.

1- control; 2- adenovirus type 12; 3- adenovirus type 3; 4- adenovirus type 6. Ordinate: number of cells.

Under aerobic conditions, the cultures of REF cells infected with adenoviruses strongly utilized glucose, in spite of the fact that under ordinary

aerobic conditions the tissue need for glucose should be lower, i.e., glycolysis is inhibited by respiration. Therefore, it was definitely interesting to thoroughly analyze the correlation of accumulated lactic acid to utilized glucose under aerobic and anaerobic conditions, since it is well known that from one molecule of glucose two molecules of lactic acid are formed.

The obtained results showed that under anaerobic conditions in the infected and control REF cultures, the utilized glucose is completely converted into lactic acid. The REF cell cultures inoculated with adenovirus type 12 almost

COMPARATIVE CHARACTERISTIC OF THE CORRELATION OF ACCUMULATED  
LACTIC ACID TO THE UTILIZED GLUCOSE IN REF CELLS 72 HOURS  
AFTER INFECTION WITH ADENOVIRUSES AND IN A-12 SARCOMA CELLS  
CULTIVATED IN VITRO UNDER AEROBIC CONDITIONS

| Cultures                | Accumulated<br>lactic acid<br>in A millimoles | Utilized<br>glucose<br>in B millimoles | Correlation<br>A:B |
|-------------------------|-----------------------------------------------|----------------------------------------|--------------------|
| Control                 | 2.56                                          | 3.6                                    | 0.71               |
| REF, infected with A-12 | 18.0                                          | 9.2                                    | 1.96               |
| REF, infected with A-3  | 8.9                                           | 8.0                                    | 1.1                |
| REF, infected with A-6  | 7.0                                           | 6.7                                    | 1.0                |
| A-12 sarcoma culture    | 14.1                                          | 9.5                                    | 1.48               |

completely converted the utilized glucose into lactic acid, whereas in REF cultures infected with adenoviruses, type 3 and type 6, the utilized glucose had not been completely converted into lactic acid. The table gives data characterizing the correlation of accumulated lactic acid to utilized glucose by cells of the REF tissue culture 72 hours after their infection with adenoviruses and utilized by cells of the A-12 sarcoma of hamsters. Evidently, the remaining part of the utilized glucose is oxidized by yet another way.

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Thus, the strong utilization of glucose and its conversion into lactic acid under aerobic conditions by cells of the REF tissue culture proves the disturbance of Pasteur's effect.

Special attention should be paid to the fact that in the cells of the hamster sarcoma culture induced by adenovirus type 12, and in the REF cultures initially contacted with oncogenic adenovirus type 12, identical changes were

observed in the glycolytic reactions (Figure 4). It is also essential to

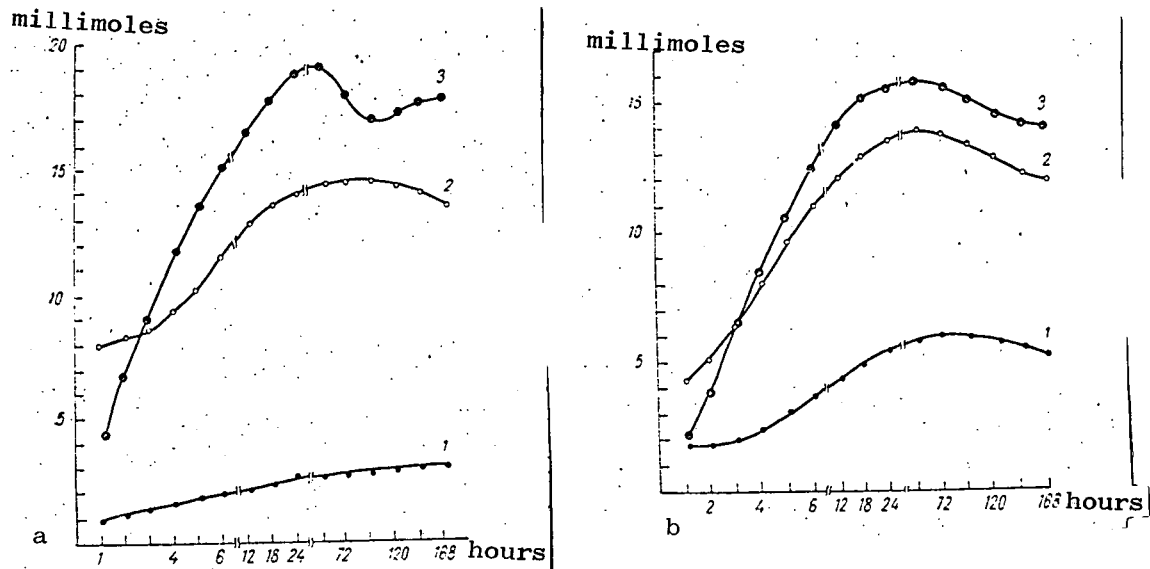


Figure 4. Aerobic (a) and anaerobic (b) glycolysis in tissue cultures. 1- in the control; 2- in A-12 sarcoma culture of hamsters; 3- in REF culture infected with adenovirus type 12. Ordinate: lactic acid in REF millimoles.

emphasize that, although in noninfected REF culture (control), aerobic glycolysis was observed, yet its level was almost three times less than in the REF cultures infected with type 12 adenovirus, and in the A-12 sarcoma cells cultivated in vitro.

Thus, on the basis of the conducted studies, it was quite obviously established that in tissue cultures of rat embryo fibroblasts initially contacted with adenoviruses, changes are observed in the glycolytic reactions. It is essential to emphasize that these changes, which arose immediately after the introduction of oncovira into the REF culture, were not connected with their reproductions. As we have observed, they monotonously remained also in the REF cell culture, where adenovirus type 12 did not propagate, and in the culture of sarcoma cells induced by type 12 adenovirus.

Possibly, the latter was conditioned by the action of oncovirus as an epigenomic factor.

#### Conclusions

1. In cultures of rat embryo fibroblasts, infected with human adenoviruses, in comparison with control, there is an increased intensity of glycolytic

reactions which depend upon the type of inoculated vira.

2. REF cell cultures, infected with adenoviruses, in comparison with the control, intensively use glucose under aerobic conditions.

3. REF cell cultures, inoculated with oncogenic virus, were characterized by more marked aerobic and anaerobic glycolysis than were REF cultures infected with type 3 and type 6 adenoviruses.

4. Identical changes were observed in the glycolytic reactions in REF cell cultures infected with type 12 adenovirus, and in the hamster sarcoma cells induced by type 12 adenovirus and cultivated in vitro.

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